(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 January 2003 (16.01.2003)

PCT

(10) International Publication Number
WO 03/004512 A1

(51) International Patent Classification[7]: C07H 21/00. C07D 275/06, 213/18, 233/58

(21) International Application Number: PCT/GB02/03029

(22) International Filing Date: 1 July 2002 (01.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/302,717 3 July 2001 (03.07.2001) US

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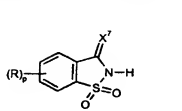
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
with international search report
before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ACTIVATORS FOR OLIGONUCLEOTIDE SYNTHESIS



(I)

(57) Abstract: A process for the synthesis of oligonucleotides using phosphoramidite chemistry is provided. The process employs as activator a 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one, preferably in the presence of an organic base. The 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one is represented by the following structural formula: (I); wherein p is 0 or an integer from 1 to 4; X^7 is O or S; R for each occurence is a substituent, preferably each independently, a halo, a substituted or unsubstituted aliphatic group, $-NR^{11}R^{12}$, $-OR^{13}$, $-OC(O)R^{13}$, $-C(O)OR^{13}$, or cyano; or two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring; R^{11} and R^{12} are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group; and R^{13} is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group. Preferred organic bases are pyridine, 3-methylpyridine, or N-methylimidazole.

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ACTIVATORS FOR OLIGONUCLEOTIDE SYNTHESIS

BACKGROUND OF THE INVENTION

Synthetic oligonucleotides are important diagnostic tools for the detection of genetic and viral diseases. In addition, oligonucleotides and modified oligonucleotides are of interest as therapeutic candidates that inhibit gene expression or protein function. Large scale synthesis of oligonucleotides for use as therapeutic candidates has become increasingly important since FDA approval of an oligonucleotide analog for the treatment of cytomegalovirus (CMV), and several other oligonucleotide analogs are currently in clinical trials. Kilogram quantities of a purified oligonucleotide analog are needed for each clinical trial.

Preparation of an oligonucleotide using phosphoramidite methodology involves condensation of a nucleoside phosphoramidite and a nucleoside or a nascent oligonucleotide. The condensation reaction (also referred to herein as the coupling reaction) requires an activator (alternatively known as a coupling agent) which facilitates the reaction. The most commonly used activator is the nucleophilic activator 1H-tetrazole. However, 1H-tetrazole is explosive and, therefore, can be hazardous to use in large scale syntheses.

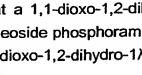
1H-tetrazole is a weak acid which protonates the trivalent phosphorus of the phosphoramidite during the first step of activation. A tetrazolide anion then displaces the dialkylamine group (e.g., N,N-diisopropyl amine) of the phosphoramidite during a second slower step to form a tetrazolyl intermediate which then reacts rapidly with the 5'-primary alcohol group of a nucleoside or a nascent oligonucleotide. When sterically hindered phosphoramidites, such as *t*-butyl-dimethylsilyl protected ribonucleoside phosphoramidites or 2'-O-methylnucleoside phosphoramidites, are used for oligonucleotide synthesis alternative activators are often needed to increase the rate of the coupling reaction. Alternative activators, such as 5-ethylthio-1H-tetrazole, 5-(p-nitrophenyl)-1H-tetrazole, and benzimidazolium triflate, are often more acidic than tetrazole and, thus, accelerate the rate of protonation of the trivalent phosphorous thereby increasing the rate of condensation.

However, since tetrazole, 5-ethylthio-1H-tetrazole, 5-(p-nitrophenyl)-1H-tetrazole, and benzimidazolium triflate are acidic, they can cause premature deprotection of the 5'-hydroxy protecting group of a phosphoramidite monomer which is typically an acid labile group. Premature deprotection can produce oligonucleotide impurities that are one base longer than the desired product (referred to herein as "N+1 impurities") and are difficult to separate from the desired product. The longer coupling times generally necessary for RNA synthesis and large scale synthesis result in an increase in premature deprotection of phosphoramidites.

Therefore, non-explosive activators that promote condensation of a nucleoside phosphoramidite with a nucleoside or a nascent oligonucleotide and which may be employed without increasing side products are needed in order to make oligonucleotides more readily available for diagnostic and therapeutic use.

SUMMARY OF THE INVENTION

It has been discovered that a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one will promote condensation of a nucleoside phosphoramidite and nucleoside monomer or a nascent oligonucleotide. The 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one can be represented by Structural Formula I:



I.

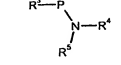
In Structural Formula I, p is 0 or an integer from 1 to 4. R for each occurrence is a substituent, preferably each independently, a halo, a substituted or unsubstituted aliphatic group, -$NR^{11}R^{12}$, -OR^{13}, -$OC(O)R^{13}$, -$C(O)OR^{13}$, cyano, a substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclyl, -CHO, -COR^{13}, -$NHCOR^{13}$, a substituted or unsubstituted aralkyl, halogenated alkyl (e.g., trifluoromethyl and trichloromethyl), or -SR^{13}. Preferably, R is halo, a substituted or unsubstituted aliphatic group, -$NR^{11}R^{12}$, -OR^{13}, -$OC(O)R^{13}$, -$C(O)OR^{13}$, or cyano. Alternatively, two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring. Preferably, the six membered ring formed is an aromatic ring. R^{11} and R^{12} are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group; or together with the nitrogen to which they are attached form a heterocyclyl group. R^{13} is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group. X^7 is O or S. Preferably, X^7 is O. It is particularly preferred that X^7 is O and p is 0.

In a preferred embodiment, a salt complex of the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and an organic base can be used to efficiently promote condensation of a nucleoside phosphoramidite and nucleoside monomer or a nascent oligonucleotide. Thus, one embodiment of the invention is a salt complex of the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one represented by Structural Formula I and an organic base.

In the presence of an organic base, 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one has good solubility particularly in organic solvents that are typically used for oligonucleotide synthesis. Therefore, another embodiment of the invention is an activator solution that includes an organic solvent, an organic base and a 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one represented by Structural Formula I. The concentration of the 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one and the organic base in the activator solution can be up to the solubility of the 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one in the solvent concerned. In a preferred embodiment, the 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one and the organic base are present in a concentration range of about 0.01 M to about 2M, for example from about 0.05M to about 0.5M. Commonly, the 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one and the organic base are present at a concentration of up to 0.25M, such as from about 0.1M to about 0.25M. In a more preferred embodiment, the 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one and the organic base are present in the same molar concentration. In a preferred embodiment, the organic solvent comprises acetonitrile. In another preferred embodiment, the organic solvent comprises an organic amide, such as dimethylformamide, 1-methyl-2-pyrrolidinone or 1,3-dimethyl-2-imidazolidinone.

In another embodiment, an oligonucleotide can be synthesized using phosphoramidite chemistry in which the coupling agent is a 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one represented by Structural Formula I. The coupling agent promotes condensation between a nucleoside or a nascent oligonucleotide having a free hydroxy or thiol group and a phosphoramidite. In a preferred embodiment, an organic base is present with the 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one during the coupling reaction. In a more preferred embodiment, the organic base is present in the same molar concentration as the 1,1-dioxo-1,2-dihydro-1λ⁶-benzo[d]isothiazol-3-one.

The nucleoside phosphoramidite can be a monomer or an oligomer, such as a dimer or a trimer. When the nucleoside phosphoramidite is a monomer it can be represented by represented by Structural Formula IIa:



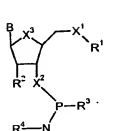
IIa.

In Structural Formula IIa, X^1 for each occurrence is, independently, -O- or -S-. Preferably, X^1 is -O- at every occurrence. X^2 for each occurrence is, independently, -O-, -S-, or -NR-. Preferably, X^2 is -O- at every occurrence. X^3 for each occurrence is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$. Preferably, X^3 is -O- at every occurrence. In a more preferred embodiment, X^1, X^2, and X^3 are all -O- at every occurrence. R^1 is an alcohol protecting group or a thio protecting group. Preferably, R^1 is an acid labile protecting group. R^2 for each occurrence is, independently, -H, -F $-OR^6$, $-NR^7R^8$, $-SR^9$, or a substituted or unsubstituted aliphatic group, such as methyl or allyl. R^3 for each occurrence is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^{10}$, $-SR^{10}$, $-O-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-O-CH_2CH_2-S(O)_2-CH_2CH_3$, $-O-CH_2CH_2-C_6H_4-NO_2$, $-S-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-S-CH_2CH_2-S(O)_2-CH_2CH_3$, or $-S-CH_2CH_2-C_6H_4-NO_2$. R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl. Alternatively, R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocyclyl group. R^6 for each occurrence is, independently, -H, a substituted or unsubstituted aliphatic group (e.g., methyl, ethyl, methoxyethyl or allyl), a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl, an alcohol protecting group, or $-(CH_2)_q-NR^{18}R^{19}$. R^7 and R^8 for each occurrence are each, independently, -H, a substituted or unsubstituted aliphatic group, or an amine protecting group. Alternatively, R^7 and R^8 taken together with the nitrogen to which they are attached are a heterocyclyl group. R^9 for each occurrence is, independently, -H, a substituted or unsubstituted aliphatic group, or a thio protecting group. R^{10} is for each occurrence is, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group or a substituted or unsubstituted aralkyl group. R^{18} and R^{19} are each, independently, -H, a substituted or unsubstituted aryl group, a substituted or unsubstituted heteroaryl group, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aralkyl group, a substituted or unsubstituted heteroaralkyl group or an amine protecting group. Alternatively, R^{18} and R^{19} taken together with the nitrogen to which they are attached form a heterocyclyl group. q is an integer from 1 to about 6. B is -H, a natural or unnatural nucleobase, protected nucleobase, protected natural or unnatural nucleobase, heterocycle or a protected heterocycle.

In another embodiment, the phosphoramidite can be an oligomer, such as a dimer or trimer. Methods of preparing and utilizing nucleoside phosphoramidite dimers and trimers in phosphoramidite synthesis of oligonucleotides are disclosed in International Patent Application No. PCT/GB01/03973, the entire teachings of which are incorporated herein by reference.

The sugar moiety of the nucleoside phosphoramidite can have either a D configuration, as in naturally occurring DNA and RNA and as in Structural Formula IIa, or

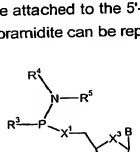
it can have an L configuration. Structural Formula IIb represents an L-nucleoside phosphoramidite:



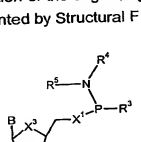
IIb

In Structural Formula IIb, X^1, X^2, X^3, R^1, R^2, R^3, R^4, R^5, and B are as defined above.

In another embodiment, the phosphoramidite group of the nucleoside phosphoramidite can be attached to the 5'-position of the sugar ring. In this embodiment, the nucleoside phosphoramidite can be represented by Structural Formulae IIIa and IIIb:



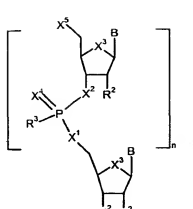
IIIa



IIIb

In Structural Formulas IIIa and IIIb, X^1, X^2, X^3, R^1, R^2, R^3, R^4, R^5, and B are as defined above.

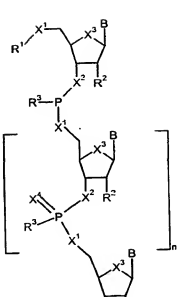
In another embodiment, the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one can be used to promote condensation of a nascent n-mer oligonucleotide (i.e., an oligonucleotide having n nucleobases) and a nucleoside phosphoramidite to form an (n+1)-mer oligonucleotide. Preferably, the nucleoside phosphoramidite can be represented by Structural Formula IIa. The nascent oligonucleotide can be represented by Structural Formula IV:



IV.

In Structural Formula IV, X^1, X^2, X^3, R^2, R^3, and B are as defined above. Each X^4 for each occurrence is, independently, O or S. X^5 for each occurrence is, independently, -OH or -SH. Preferably, X^5 is -OH. R^{16} is a hydroxy protecting group, a thio protecting group, an amino protecting group, $-(CH_2)_q-NR^{18}R^{19}$, a solid support, or a cleavable linker attached to a solid support, such as a group of the formula $-Y^2-L-Y^2-R^{15}$. Y^2 for each occurrence is, independently, a single bond, -C(O)-, $-C(O)NR^{17}-$, -C(O)O-, $-NR^{17}-$ or -O-. L is a linker which is preferably a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group. More preferably, L is an ethylene group. R^{17} is -H, a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group. R^{15} is any solid support suitable for solid phase oligonucleotide synthesis known to those skilled in the art. Examples of suitable solid supports include controlled-pore glass, polystyrene, microporous polyamide, such as poly(dimethylacrylamide), and polystyrene coated with polyethylene. In many embodiments, R^{16} represents a cleavable linker, such as a succinyl or oxaloyl linker, attached to a solid support. n is zero or a positive integer.

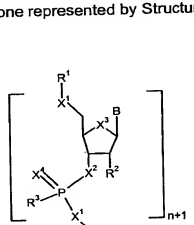
The nascent oligonucleotide is contacted with the phosphoramidite and a 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one represented by Structural Formula I. In a preferred embodiment, an organic base is also present when the nascent oligonucleotide is contacted with the 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one. More preferably, the organic base is present in the same molar concentration as the 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one. The nascent oligonucleotide reacts with the phosphoramidite to form an (n+1) oligonucleotide having a 5'-terminal trivalent phosphorous linkage represented by Structural Formula V:



V.

In Structural Formula V, X^1, X^2, X^3, X^4, R^1, R^2, R^3, R^{16}, B and n are defined as above.

The oligonucleotide represented by Structural Formula V can then be contacted with an oxidizing agent or a sulfurizing agent to form an oligonucleotide having a pentavalent phosphorous backbone represented by Structural Formula VI:



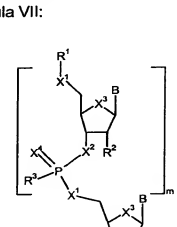
VI.

In Structural Formula VI, X^1, X^2, X^3, X^4, R^1, R^2, R^3, R^{16}, B and n are defined as above.

After oxidizing or sulfurizing the (n+1) oligonucleotide, X^5 groups which did not react with the phosphoramidite can be capped by conventional capping techniques known

in the art. For example, the unreacted X^5 groups can be reacted with an acid chloride or an anhydride in the presence of a base. Typically, X^5 groups are capped with acetyl chloride or acetic anhydride in pyridine.

After the oxidation or sulfurization step or after the capping step, the (n+1) oligonucleotide can be deprotected by reacting it with a reagent to remove R^1. If R^1 is an acid labile protecting group, the (n+1) oligonucleotide is treated with an acid to remove R^1. If R^1 is a trialkylsilyl group, such as *t*-butyldimethylsilyl group or a triisopropylsilyl group, the (n+1) oligonucleotide can be treated with fluoride ions to remove R^1. Typically, *t*-butyldimethylsilyl and a triisopropylsilyl are removed by treatment with a solution of tetrabutylammonium fluoride in THF or with hydrogen fluoride and a conjugate base, such as $(C_2H_5)_3N.3HF$. Methods for removing *t*-butyldimethylsilyl can be found in Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., pages 77-83, the teachings of which are incorporated herein by reference in their entirety. The above reaction steps, or reaction cycle, can be repeated one or more times to form an oligonucleotide of the desired length. When it is desired to obtain an oligonucleotide product in which the 5'-end group is protected, the final step of the reaction cycle can be the capping step, if a capping step is done, or the final step of the reaction can be an oxidation or sulfurization step if a capping step is not done. When the oxidation or sulfurization step or the capping step is the final step, the oligonucleotide can be represented by Structural Formula VII:

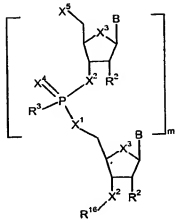


VII.

In Structural Formula VII, X^1, X^2, X^3, X^4, R^1, R^2, R^3, R^{16}, and B are defined as above. m is an integer.

Alternatively, the final step of the reaction cycle can be removal of R^1 if it is desired to obtain an oligonucleotide which does not have a 5'-protecting group. When removal of

R^1 is the final reaction step, the oligonucleotide can be represented by Structural Formula VIII:



VIII.

In Structural Formula VIII, X^1, X^2, X^3, X^4, X^5, R^1, R^2, R^3, R^{16}, B and m are defined as above.

Oligonucleotides produced by the method of the present invention can be deprotected, and as appropriate cleaved from a solid support, using methods known in the art for the given protecting groups and/or solid support.

1,1-Dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-ones in the presence of an organic base promote phosphoramidite condensation reactions with at least equal efficiency as tetrazole. However, fewer undesirable side products are produced when a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one is used instead of tetrazole. In addition, the complexes of the invention are non-explosive and therefore, safer to use than tetrazole particularly in large scale synthesis of oligonucleotides.

DETAILED DESCRIPTION OF THE INVENTION

Aliphatic groups, as used herein, include straight chained or branched C_1-C_{18} hydrocarbons which are completely saturated or which contain one or more unconjugated double bonds, or cyclic C_3-C_{18} hydrocarbons which are completely saturated or which contain one or more unconjugated double bonds. Alkyl groups are straight chained or branched C_1-C_8 hydrocarbons or C_3-C_8 cyclic hydrocarbons which are completely saturated. Aliphatic groups are preferably alkyl groups.

Aryl groups include carbocyclic aromatic ring systems (e.g., phenyl) and carbocyclic aromatic ring systems fused to one or more carbocyclic aromatic (e.g., naphthyl and anthracenyl) or an aromatic ring system fused to one or more non-aromatic ring (e.g., 1,2,3,4-tetrahydronaphthyl).

Heterocyclic groups, as used herein, include heteroaryl groups and heteroalicyclyl groups. Heteroaryl groups, as used herein, include aromatic ring systems that have one or more heteroatoms selected from sulfur, nitrogen or oxygen in the aromatic ring. Preferably, heteroaryl groups are five or six membered ring systems having from one to four heteroatoms. A heteroalicyclyl group, as used herein, is a non-aromatic ring system that preferably has five to six atoms and includes at least one heteroatom selected from nitrogen, oxygen, and sulfur. Examples of heterocyclic groups include morpholinyl, piperidinyl, piperazinyl, thiomorpholinyl, pyrrolidinyl, thiazolidinyl, tetrahydrothienyl, azetidinyl, tetrahydrofuryl, dioxanyl and dioxepanyl thienyl, pyridyl, thiadiazolyl, oxadiazolyl, indazolyl, furans, pyrroles, imidazoles, pyrazoles, triazoles, pyrimidines, pyrazines, thiazoles, isoxazoles, isothiazoles, tetrazoles, oxadiazoles, benzo(b)thienyl, benzimidazole, indole, tetrahydroindole, azaindole, indazole, quinoline, imidazopyridine, purine, pyrrolo[2,3-d]pyrimidine, and pyrazolo[3,4-d]pyrimidine.

Azaheterocyclyl compounds, as used herein, include heteroaryl groups which have one or more nitrogen atom in the aromatic ring and heteroalicyclyl groups that have at least one nitrogen atom in the non-aromatic ring system. Preferably, azaheteroaryl compounds have five- or six-membered aromatic rings with from one to three nitrogens in the aromatic ring. Preferably, azaheteroalicyclyl compounds are five- or six-membered rings, commonly comprising one or two nitrogens in the ring. Preferred azaheterocyclyl compounds are organic bases. Examples of azaheterocyclyl compounds that are organic bases include pyrimidines, 1-alkylpyrazoles, especially 1-(C_{1-4} alkyl)pyrazoles, 1-arylpyrazoles, 1-benzylpyrazoles, pyrazines, N-alkylpurines, especially N-(C_{1-4} alkyl)purines, N-arylpurines, N-benzylpurines, N-alkylpyrroles, especially N-(C_{1-4} alkyl)pyrroles, N-arylpyrroles, N-benzylpyrroles, pyridines, N-alkylimidazoles, especially N-(C_{1-4} alkyl)imidazoles, N-arylimidazoles, especially N-phenylimidazole, N-benzylimidazoles, quinolines, isoquinolines, quinoxalines, quinazolines, N-alkylindoles, especially N-(C_{1-4} alkyl)indoles, N-arylindoles, N-benzylindoles, N-alkylbenzimidazoles especially N-(C_{1-4} alkyl)benzimidazoles, N-arylbenzimidazoles, N-benzylbenzimidazoles, triazine, thiazole, 1-alkyl-7-azaindoles, especially 1-(C_{1-4} alkyl)–7-azaindoles, 1-aryl-7-azaindoles, 1-benzyl-7-azaindoles, pyrrolidines, morpholines, piperidines, and piperazines. Especially preferred azaheterocyclyl compounds are pyridines, such as pyridine and 3-methylpyridine, and N-(C_{1-4} alkyl) imidazoles, such as N-methylimidazole.

An aralkyl group, as used herein, is an aromatic substituent that is linked to a moiety by an alkyl group. Preferred aralkyl groups include benzyl groups.

A heteroaralkyl group, as used herein, is a heteroaryl substituent that is linked to a moiety by an alkyl group.

An organic base is an organic compound that has a tendency to accept protons at pH 7. Preferred organic bases are secondary amines, tertiary amines or azaheterocyclyl compounds, each of which may be substituted or unsubstituted by one or more

substituents. An aprotic organic base is an organic base that has no hydrogen bonding protons in its chemical structure before accepting a proton. Aprotic organic bases such as tertiary amines and aprotic azaheterocyclyl compounds are preferably used in conjunction with 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-ones, as described herein, to promote condensation reactions.

Tertiary amines are organic bases that have a nitrogen atom which is bonded to three carbon atoms, often to three aryl, commonly phenyl, and/or alkyl groups, commonly to three alkyl groups, including for example trialkylamines such as trimethylamine, triethylamine, and diisopropylethylamine. In addition, tertiary amines can be azaheterocyclyl groups wherein the nitrogen atom is aprotic. Tertiary amines that are azaheterocyclyl groups are preferred. Examples of azaheterocyclyl tertiary amines are N-alkylpyrrolidines, N-arylpyrrolidines, N-alkylpyrroles, N-arylpyrroles, N-alkylmorpholines, N-arylmorpholines, N-alkylpiperidines, N-arylpiperidines, N,N-dialkylpiperazines, N,N-diarylpiperazines, N-alkyl-N-aryl-piperazines, quinuclidines, and 1,8-diazabicyclo[5.4.0]undec-7-enes. Tertiary amines can also be azaheteroaryl or azaheteroalicyclyl compounds.

Secondary amines are organic bases comprising a nitrogen bonded to a single hydrogen and to two carbon atoms. Commonly the nitrogen atom is bonded to two alkyl or aryl groups or forms part of an azaheterocyclic group. Examples of secondary amine compounds include diethylamine and diisopropylamine.

Suitable substituents for aliphatic groups, aryl groups, aralkyl groups, heteroaryl groups, azaheteroaryl groups and heteroalicyclyl groups include aryl groups, halogenated aryl groups, alkyl groups, halogenated alkyl (e.g. trifluoromethyl and trichloromethyl), aliphatic ethers, aromatic ethers, benzyl, substituted benzyl, halogens, particularly chloro and fluoro groups, cyano, nitro, -S-(aliphatic or substituted aliphatic group), and -S-(aromatic or substituted aromatic).

Amine, hydroxy and thiol protecting groups are known to those skilled in the art. For examples of amine protecting groups see Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., pages 309-405, the teachings of which are incorporated herein by reference in their entirety. Preferably, amines are protected as amides or carbamates. For examples of hydroxy protecting groups see *Id.*, pages 10-142, the teachings of which are incorporated herein by reference in their entirety. A preferred hydroxy protecting group is *t*-butyldimethylsilyl group. For examples of thiol protecting groups see *Id.*, pages 277-308, the teachings of which are incorporated herein by reference in their entirety.

An acid labile protecting group is a protecting group which can be removed by contacting the group with a Bronsted or a Lewis acid. Acid labile protecting groups are known to those skilled in the art. Examples of common acid labile protecting groups include substituted or unsubstituted trityl groups (*Id.*, pages 60-62), substituted or

unsubstituted tetrahydropyranyl groups (*Id.*, pages 31-34), substituted or unsubstituted tetrahydrofuranyl groups (*Id.*, pages 36-37) or pixyl groups (*Id.*, page 65). Trityl groups are commonly substituted by electron donating substituents such as alkoxy groups. A preferred acid labile protecting group is a substituted or unsubstituted trityl, for example 4,4'-dimethoxytrityl (hereinafter "DMT").

Nucleoside bases include naturally occurring bases, such as adenine, guanine, cytosine, thymine, and uricil and modified bases such as 7-deazaguanine, 7-deaza-8-azaguanine, 5-propynylcytosine, 5-propynyluricil, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-6-oxopurine, 6-oxopurine, 3-deazaadenosine, 2-oxo-5-methylpyrimidine, 2-oxo-4-methylthio-5-methylpyrimidine, 2-thiocarbonyl-4-oxo-5-methylpyrimidine, 4-oxo-5-methylpyrimidine, 2-amino-purine, 5-fluorouricil, 2,6-diaminopurine, 8-aminopurine, 4-triazolo-5-methylthymine, and 4-triazolo-5-methyluricil.

A protected nucleoside base is a nucleoside base in which reactive functional groups of the base are protected. Similarly, a protected heterocycle is a heterocycle in which reactive substitutents of the heterocycle are protected. Typically, nucleoside bases or heterocycles have amine groups which can be protected with an amine protecting group, such as an amide or a carbamate. For example, the amine groups of adenine and cytosine are typically protected with benzoyl protecting groups, and the amine groups of guanine is typically protected with an isobutyryl group, an acetyl group or t-butylphenoxyacetyl group. However, other protection schemes may be used. For example, for fast deprotection, the amine groups of adenine and guanine are protected with phenoxyacetyl groups and the amine group of cytosine is protected with an isobutyryl group or an acetyl group. Conditions for removal of the nucleobase or heterocycle protecting group will depend on the protecting group used. When an amide protecting group is used, it can be removed by treating the oligonucleotide with a base solution, such as a concentrated ammonium hydroxide solution, *n*-methylamine solution or a solution of *t*-butylamine in ammonium hydroxide.

The term "oligonucleotide," as used herein, includes naturally occurring oligonucleotides, for example 2'-deoxyribonucleic acids (hereinafter "DNA") and ribonucleic acids (hereinafter "RNA") and nucleic acids containing modified sugar moieties, modified phosphate moieties, or modified nucleobases. Modification to the sugar moiety includes replacing the ribose ring with a hexose, cyclopentyl or cyclohexyl ring. Alternatively, the D-ribose ring of a naturally occurring nucleic acid can be replaced with an L-ribose ring or the b-anomer of a naturally occurring nucleic acid can be replaced with the a-anomer. The oligonucleotide may also comprise one or more abasic moieties. Modified phosphate moieties include phosphorothioates, phosphorodithioates, methyl phosphonates, methyl phosphates, and phosphoramidates. Such nucleic acid analogs are known to those of skill in the art. Oligonuceotides comprising mixtures of two or more of the foregoing may be prepared, for example, oligonuceotides comprising mixtures of

deoxyribo- and ribonucleosides, particularly mixtures of deoxyribonucleosides and 2'-O-substituted ribonucelosides, such as 2'-O-methyl or 2'-O-methoxyethyl ribonucleosides. Examples of oligonucleotides comprising mixtures of nucleosides include ribozymes.

A chimeric oligonucleotide is an oligonucleotide that has both phosphodiester and phosphorothioate linkages.

A synthetic oligonucleotide preferably has from 2 to about 100 nucleobases. More preferably, a synthetic oligonucleotide has 2 to about 75 nucleobases. Many synthetic oligonucleotides of current therapeutic interest comprise from 8 to 40 nucleobases.

The synthesis of the oligonucleotide can be done in solution or on a solid support. When the synthesis is in solution, R^{16} is an alcohol, amine or thiol protecting group. After synthesis of the oligonucleotide the alcohol, amine or thiol protecting group can be removed. When the oligonucleotide is synthesized on a solid support, R^{16} represents a solid support or preferably a cleavable linker attached to a solid support, such as a group of formula $-Y_2-L-Y_2-R^{15}$. In general, the solution phase synthesis or the solid phase synthesis of oligonucleotides using a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one compound instead of tetrazole to promote condensation of a nascent oligonucleotide and a phosphoramidite monomer is carried out similar to method which have been developed for synthesis of oligonucleotides using tetrazole as an activator. Examples of typical conditions for solution phase synthesis and solid phase synthesis oligonucleotides using a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one compound to promote the condensation reaction are set forth below.

The first step of preparing the oligonucleotide involves coupling a nucleoside phosphoramidite, such as the phosphoramidite represented by Structural Formula IIa, with a nucleoside or nascent oligonucleotide that has a free hydroxy or thiol group, such as a 5-deprotected nucleoside or nascent oligonucleotide represented by Structural Formula IV. During the coupling reaction, the hydroxy or thiol group of the nucleoside or nascent oligonucleotide reacts with the nucleoside phosphoramidite by displacing the $-NR^4R^5$ group. When the synthesis is done in solution, the nucleoside or nascent oligonucleotide is often present in a concentration of about 0.001 M to about 1.0 M, and preferably the nucleoside or nascent oligonucleotide is present in a concentration of about 0.025 M to about 0.5 M. The nucleoside phosphoramidite is preferably present in a concentration of about 1.1 equivalents to about 2 equivalents with respect to the nucleoside or nascent oligonucleotide. From about 0.5 equivalents, often from about 2.5 equivalents, to about 5.0 equivalents, with respect to the nucleoside or nascent oligonucleotide, of a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one is added to promote the condensation reaction. Preferably, the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one is added as a salt complex with an organic base, such as a pyridinium salt, a 3-picolinium salt or an N-methylimidazolium salt. The reaction time is commonly about 20 min. to about 60 min., and an (n+1) nascent oligonucleotide with a terminal trivalent phosphorous linkage is

formed, such as the nascent oligonucleotide represented by Structural Formula V.

A second step of preparing an oligonucleotide involves oxidizing or sulfurizing the terminal trivalent phosphorous group of the nascent oligonucleotide. In a solution phase synthesis, the oxidation reaction is often carried out by treating the oligonucleotide with an oxidizing agent such as I_2 in the presence of water or a peroxide such as *t*-butyl hydrogen peroxide in an organic solvent. When I_2 and water are used, the oxidizing solution typically contains about 1.1 to about 1.8 equivalents of I_2 in the presence of a base and a trace amount of water. The reaction is carried out in an aprotic polar solvent, such as THF, combined with a base, such as a tertiary alkylamine and about 1% water. The ratio of aprotic solvent to base is about 4 : 1 (vol./vol.) to about 1 : 4 (vol./vol.). After about 5 min. to about 20 min., the reaction mixture is poured into an aqueous solution of sodium bisulfite to quench the excess iodine, then extracted into an organic solvent.

Alternatively, the terminal trivalent phosphorous group can be sulfurized using any sulfur transfer reagent known to those skilled in the art of oligonucleotide synthesis. Examples of sulfur transfer reagents include *3H*-benzodithiol-3-one 1,1-dioxide (also called "Beaucage reagent"), dibenzoyl tetrasulfide, phenylacetyl disulfide, N,N,N',N'-tetraethylthiuram disulfide, elemental sulfur, and 3-amino-[1,2,4]dithiazole-5-thione (see U.S. Patent No. 6,096,881, the entire teachings of which are incorporated herein by reference). Reaction conditions for sulfurization of an oligonucleotide using the above reagents can be found in Beaucage, *et al.*, *Tetrahedron* (1993), *49*:6123, the teachings of which are incorporated herein by reference in their entirety. 3-Amino-[1,2,4]dithiazole-5-thione is a preferred sulfur transfer reagent. Generally, an oligonucleotide is contacted with a solution of 3-amino-[1,2,4]dithiazole-5-thione in an organic solvent, such pyridine/acetonitrile (1:9) mixture or pyridine, having a concentration of about 0.05 M to about 0.2 M. The sulfurization reaction is commonly complete in about 30 sec. to about 2 min.

After oxidation or sulfurization of the oligonucleotide, any unreacted free hydroxy or thiol groups can be capped so that they cannot react in subsequent coupling steps. Capping failure sequences allows them to be more readily separated from full length oligonucleotide product. Any reagent which will react with a hydroxy or thiol group and prevent it from reacting with a phosphoramidite can be used as a capping reagent. Typically, an anhydride, such as acetic anhydride or isobutyric anhydride, or an acid chloride, such as acetyl chloride or isobutyryl chloride, in the presence of a base is used as a capping reagent.

After the capping reaction is complete, the R^1 protecting group is removed. When R^1 is an acid labile protecting group, R^1 is removed by treating the oligonucleotide with an acid. Preferably, R^1 is a trityl group, such as 4,4'-dimethoxytrityl. When the R^1 is a trityl group, it can be removed by treating the oligonucleotide with a solution of dichloroacetic acid or trichloroacetic acid in an organic solvent, such as dichloromethane or toluene.

Once the R^1 protecting group has been removed, the reaction cycle (i.e., coupling step, oxidation or sulfurization step, capping step (optional) and deprotection step) optionally can be repeated one or more times to obtain an oligonucleotide of the desired length.

A chimeric oligonucleotide can be prepared by oxidizing the terminal trivalent phosphorous group in one or more reaction cycles and sulfurizing the terminal trivalent phosphorous group in one or more different reaction cycles. Alternatively, a chimeric oligonucleotide can be prepared by selecting phosphoramidite monomers in which some of the R^3 groups are protected hydroxyl groups, such as $-OCH_2CH_2CN$, and some of the R^3 groups are protected thiol groups, such as $-SCH_2CH_2CN$. In this method, the oligonucleotide is oxidized after the coupling step in each reaction cycle.

When it is desired to obtain an oligonucleotide product in which the R^1 group remains, the final step of the reaction cycle can be the capping step, if a capping step is done, or the final step of the reaction can be an oxidation or sulfurization step if a capping step is not done. If an R^1 deprotected oligonucleotide is desired, the reaction cycle can end with the deprotection step. Usually, an R^1 protected oligonucleotide is the desired product if the oligonucleotide is to be purified by reverse phase high performance liquid chromatography (HPLC). If the oligonucleotide is to be purified by ion-exchange chromatography or electophoresis, an R^1 deprotected oligonucleotide is usually the desired product.

The solid phase synthesis of an oligonucleotide using a 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one and, preferably, an organic base to promote condensation of a nucleoside phosphoramidite with a support bound nucleoside or nascent oligonucleotide having a free hydroxy group of thiol group generally utilizes the same reaction cycle and reagents as the solution phase synthesis. Commonly, the nucleoside is first loaded on the solid support to the maximum suitable for the particular resin used. For example, loading can be about 50 µmole to about 700 µmole per gram of support. In the condensation step, a solution of nucleoside phosphoramidite, typically having a concentration of about 0.01 M to about 1 M, preferably about 0.1 M, in an organic solvent, such as acetonitrile, is reacted with the support bound nucleoside to form a nascent oligonucleotide having a terminal trivalent phosphorous linkage. If a nucleoside phosphoramidite represented by either Structural Formula IIa or IIb is used, the nascent oligonucleotide will have a 5'-terminal trivalent phosphorous linkage after completion of the coupling reaction. If a nucleoside phosphoramidite represented by either Structural Formula IIIa or IIIb is used, the nascent oligonucleotide will have a 3'-terminal trivalent phosphorous linkage after completion of the coupling reaction. Preferably, the nucleoside phosphoramidites used can be represented by Structural Formula IIa. A solution of the 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one having a concentration of about 0.015M to about 1.5 M, often from about 0.05M to about 0.5M, preferably from 0.1 to 0.25M, is usually mixed with the solution containing the phosphoramidite monomer just prior to or during the condensation

reaction. Preferably, an organic base is also present in the solution at a concentration of about 0.015M to about 1.5 M, often from about 0.05M to about 0.5M, preferably from 0.1 to 0.25M. Preferably, the organic base is present in the same molar concentration as the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one. The 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one may be employed at a mole ratio to nucleoside phosphoramidite which is catalytic, that is sub-stoichiometric, or at a mole ratio which is stoichiometric or greater than stoichiometric. In many embodiments, the mole ratio of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one to nucleoside phosphoramidite is in the range of from about 0.2 : 1 to 5 : 1, often from 0.25 : 1 to 4 : 1, preferably from about 0.3 : 1 to 2 : 1, for example about 1 : 1. Then the support bound 5'-deprotected nucleoside is contacted with the mixture for about 2 min. to about 10 min., preferably about 5 min.

If the terminal trivalent phosphorous linkage is to be oxidized after the coupling reaction is complete, the solid support containing the nascent oligonucleotide is contacted with an oxidizing agent such as a mixture of I_2 and water or a peroxide such as *t*-butyl hydroperoxide in an organic solvent such as acetonitrile or toluene. A mixture of I_2 and H_2O is a preferred oxidizing reagent. When a mixture of I_2 and water is used other water miscible organic solvents can also be present. Typically, the solid support bound oligonucleotide containing trivalent phosphorous internucleotide linkages can be contacted with a solution of I_2 in a solvent mixture of water, an aprotic, water miscible solvent, and a base. An example of a typical oxidation solution is about 0.05 M to about 1.5 M I_2 in a solution of (2 : 80 : 20) water/tetrahydrofuran/lutidine (vol./vol./vol.). The solid support is typically treated with the I_2 solution for about 30 seconds to about 1.5 min.

Alternatively, the solid support bound nascent oligonucleotide can be contacted with a solution of a sulfur transfer reagent in an organic solvent to sulfurize the trivalent phosphorous groups. For example, the support bound oligonucleotide can be contacted with a solution of 3-amino-[1,2,4]-dithiazole-5-thione (about 0.05 M-0.2 M) in an organic solvent, such as acetonitrile or pyridine, for about 30 sec. to about 2 min.

In solid phase oligonucleotide synthesis, the solid support bound nascent oligonucleotide optionally can be contacted with a solution of the capping reagent for about 30 sec. to about 1 min. Following the capping step, the deprotection step is accomplished by contacting the support bound oligonucleotide with an acid solution for about 1 min. to about 3 min. The reaction cycle can optionally be repeated one or more times until an oligonucleotide of the desired length is synthesized. As in the solution phase synthesis, an R^1 protected oligonucleotide is obtained when the reaction cycle ends with either the capping step or the oxidation or sulfurization step. An R^1 deprotected oligonucleotide is obtained when the reaction cycle is ended with the deprotection step.

When the solid phase synthesis is completed, the oligonucleotide can be removed from the solid support by standard methods. Generally, the solid support is treated with a solution of concentrated ammonium hydroxide at 25°C-60°C for about 0.5 hours to about

16 hours or longer depending on the oligonucleotide sequence and whether it is desired to remove the nucleobase protecting groups during this step. The oligonucleotides are advantageously purified by methods known in the art, such as one or more of ion-exchange chromatography, reverse phase chromatography, and precipitation from an appropriate solvent. Further processing of the product by for example ultrafiltration may also be employed.

A particularly preferred aspect of the present invention comprises a method for the synthesis of an oligonucleotide comprising coupling a nucleoside phosphoramidite, preferably a nucleoside 3'-phosphoramidite, with a nucleoside or nascent oligonucleotide comprising a free hydroxy group, preferably a free 5'-hydroxy group, in the presence of an activator, wherein the activator comprises a mixture of a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and an N-alkylimidazole, preferably N-methylimidazole.

In this particularly preferred embodiment, the phosphoramidite commonly comprises a moiety of formula $-P(OCH_2CH_2CN)N(CH(CH_3)_2)_2$. Commonly, in this embodiment, the concentration of each of the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and N-alkylimidazole is from 0.1 to 0.25M, and preferably the mole ratio of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one to N-alkylimidazole is about 1 : 1 to about 1 : 1.5 : 1, most preferably 1 : 1. In this particularly preferred embodiment, the mole ratio of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one to phosphoramidite is preferably from 0.5 : 1 to 2 : 1.

The present invention is illustrated without limitation by the following Examples.

Example 1: Preparation a Salt Complex of 1,1-Dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and Pyridine

1,1-Dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one was suspended in acetonitrile, and 1.1 eq. of pyridine with respect to the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one was added dropwise to the suspension. The solution turned clear at the end of the addition, and a salt complex of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and pyridine separated out of the solution as a fine crystalline material. The crystals were washed with either ether or hexane to remove traces of pyridine and acetonitrile ^{1}H NMR (DMSO) chemical shifts in ppm: 8.8 (2H, s), 8.2 (1H, q), 8.0 (1H, q) and 7.6-7.9 (6H, m).

Example 2: Preparation a Salt Complex of 1,1-Dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and 3-Picoline

A salt complex of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and 3-picoline was prepare in the same manner as described in Example 1. ^{1}H NMR (DMSO) chemical shifts in ppm: 8.8 (1H, s), 8.72 (1H, d), 8.27 (1H, d), 8.0 (2H, d), 7.77-7.93 (6H,

m) and 2.45 (3H, s).

Example 3: Preparation a Salt Complex of 1,1-Dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and N-Methylimidazole

1,1-Dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one was suspended in acetonitrile, and 1.1 eq. of N-methylimidazole with respect to the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one was added dropwise to the suspension. The reaction mixture was concentrated under reduced pressure to form the crystalline salt which was washed with either ether or hexane to remove traces of N-methylimidazole and acetonitrile. ^{1}H NMR (DMSO) chemical shifts in ppm: 13.9 (1H, s), 9.03 (1H, s), 7.59-7.75 (6H, m) and 3.88 (3H, s),

Example 4: Synthesis of deoxyribo-oligonucleotides using a salt complex of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and an organic Base.

Synthesis of the oligonucleotide was carried out on DNA synthesizer Oligo Pilot II (Amersham Pharmacia Biotech). The standard phosphoramidite chemistry protocol was followed for the synthesis with slight modifications. The concentration of phosphoramidite monomers was 0.1 M in acetonitrile. The salt complex of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and pyridine, 3-picoline or N-methylimidazole was used in place of tetrazole as the activator during the condensation step. The concentration of the salt complex was 0.25 M in acetonitrile. The coupling time used for the chain elongation using the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one salt complex were similar to coupling times used when tetrazole is activator. After the condensation step, the phosphite triester linkage was converted either to stable phosphate triester with iodine solution or to stable phosphorothioate triester with Beaucage reagent or 3-amino-1,2,4,-dithiazole-5-thione. At the end of the synthesis, solid supports linked with fully protected oligonucleotide were treated with 10% *t*-butylamine in concentrated ammonium hydroxide for 16-20 hr at 50°C in order to release the oligonucleotide and to remove the β-cyanoethyl protecting groups and the nucleobase protecting groups. The crude oligonucleotides were analyzed by ion exchange HPLC, capillary electrophoresis and MALDI-TOF mass spectrometry and were compared to oligonucleotides prepared using tetrazole as the activator. Table 1 describes the conditions used to synthesize phosphorothioate oligonucleotide sequence 5' TCT-CCC-AGC-GTG-CGC-CAT 3' (SEQ ID NO 1), and Table 2 describes the results obtained from the various syntheses. The salt complex illustrated in Tables 1 and 2 is 1, 1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and N-methylimidazole.

Table 1: Synthesis Parameters for synthesis of SEQ ID NO 1.

Solid Support	Scale of synthesis	Activator	Molar equiv. of Amidite	Activator vs. Amidite	Equiv. of sulfurizing agent
CPG-beads	746µmole	Tetrazole	2.0 equ.	4.3	3.2
CPG-beads	737µmole	salt-complex	2.0 equ.	4.0	3.3
CPG-beads	737µmole	salt-complex	1.5 equ.	3.3	3.3
Rigid PS	626µmole	salt-complex	2.0 equ.	4.0	3.8
Rigid PS	600µmole	Tetrazole	2.0 equ.	4.3	4.0

Table 2: Analysis and results of SEQ ID NO 1.

Solid supports	Scale	Activator	mol equ. of amidite	Total OD units	FLP by CGE	FLP by HPLC	Mol. Wt.
CPG beads	746µmol	Tetrazole	2.0 equ.	84504	74%	77%	5688
CPG beads	737µmol	Com Salt	2.0 equ.	82134	77%	79%	5687
CPG beads	737µmol	Com. Salt	1.5 equ.	82320	77%	79%	5689
Rigid PS	626µmol	Com. Salt	2.0 equ.	80712	76%	76%	5686
Rigid PS	600µmol	Tetrazole	2.0 equ.	75006	73%	71%	5687

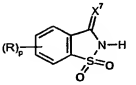
FLP = full length product, CGE = capillary gel electrophoresis, HPLC = Ion exchange HPLC

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A salt complex comprising an organic base and a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one represented by the following structural formula:



wherein:

p is 0 or an integer from 1 to 4;

X^7 is O or S; and

R for each occurrence is a substituent.

2. The complex of Claim 1, wherein each R independently is halo, a substituted or unsubstituted aliphatic group, $-NR^{11}R^{12}$, $-OR^{13}$, $-OC(O)R^{13}$, $-C(O)OR^{13}$, cyano, a substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclyl, -CHO, $-COR^{13}$, $-NHCOR^{13}$, a substituted or unsubstituted aralkyl, or $-SR^{13}$; or two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring; wherein:

R^{11} and R^{12} are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group; or together with the nitrogen to which they are attached form a heterocyclyl group; and

R^{13} is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group.

3. The complex of Claim 1 or Claim 2, wherein the organic base is an amine.

4. The complex of Claim 3, wherein the amine is a tertiary amine.

5. The complex of Claim 4, wherein the tertiary amine is selected from the group consisting of a trialkylamine, a substituted or unsubstituted N-alkylpyrrolidine, a substituted or unsubstituted N-arylpyrrolidine, a substituted or unsubstituted N-alkylpyrrole, a substituted or unsubstituted N-arylpyrrole, a substituted or unsubstituted N-alkylmorpholine, a substituted or unsubstituted N-arylmorpholine, a substituted or unsubstituted N-alkylpiperidine, a substituted or unsubstituted N-arylpiperidine, a

substituted or unsubstituted N,N-dialkylpiperazine, a substituted or unsubstituted N,N-diarylpiperazine, a substituted or unsubstituted N-alkyl-N-arylpiperazine, substituted or unsubstituted quinuclidine, and a substituted or unsubstituted 1,8-diazabicyclo[5.4.0]undec-7ene.

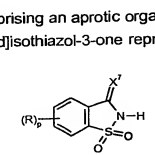
6. The complex of Claim 3, wherein the amine is a substituted or unsubstituted azaheterocyclyl compound.

7. The complex of Claim 6, wherein the azaheterocyclyl compound is selected from the group consisting of a substituted or unsubstituted pyrimidine, a substituted or unsubstituted 1-alkylpyrazole, a substituted or unsubstituted 1-arylpyrazole, a substituted or unsubstituted pyrazine, a substituted or unsubstituted N-alkylpurine, a substituted or unsubstituted N-arylpurine, a substituted or unsubstituted N-alkylpyrrole, a substituted or unsubstituted N-arylpyrrole, a substituted or unsubstituted pyridine, a substituted or unsubstituted N-alkylimidazole, a substituted or unsubstituted N-arylimidazole, a substituted or unsubstituted quinoline, a substituted or unsubstituted isoquinoline, a substituted or unsubstituted quinoxaline, a substituted or unsubstituted quinazoline, a substituted or unsubstituted N-alkylindole, a substituted or unsubstituted N-arylindole, a substituted or unsubstituted N-alkylbenzimidazole, a substituted or unsubstituted N-arylbenzimidazole, a substituted or unsubstituted triazine, a substituted or unsubstituted thiazole, a substituted or unsubstituted 1-alkyl-7-azaindole, a substituted or unsubstituted 1-aryl-7-azaindole, a substituted or unsubstituted pyrrolidine, a substituted or unsubstituted morpholine, a substituted or unsubstituted piperidine, and a substituted or unsubstituted piperazine.

8. The complex of Claim 7, wherein the azaheterocyclyl compound is pyridine, 3-methylpyridine, or N-methylimidazole.

9. A complex according to any one of Claims 1 to 8, wherein X^7 is O and p is 0.

10. An activator solution comprising an aprotic organic solvent, an organic base and a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one represented by the following structural formula:



wherein:

p is 0 or an integer from 1 to 4;

X^7 is O or S; and

R for each occurrence is a substituent.

11. The activator solution of Claim 10, wherein each R independently is halo, a substituted or unsubstituted aliphatic group, $-NR^{11}R^{12}$, $-OR^{13}$, $-OC(O)R^{13}$, $-C(O)OR^{13}$, cyano, a substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclyl, -CHO, $-COR^{13}$, $-NHCOR^{13}$, a substituted or unsubstituted aralkyl, or $-SR^{13}$; or two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring; wherein:

R^{11} and R^{12} are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group; or together with the nitrogen to which they are attached form a heterocyclyl group; and

R^{13} is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group.

12. The activator solution of Claim 10 or 11, wherein the organic solvent is acetonitrile.

13. The activator solution of Claim 10 or 11, wherein the organic solvent is an organic amide.

14. The activator solution of Claim 13, wherein the organic amide is selected from the group consisting of dimethylformamide, 1-methyl-2-pyrrolidinone, and 1,3-dimethyl-2-imidazolidinone.

15. The activator solution of any one of Claims 10 to 14, wherein the 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one is present in a concentration of about 0.01 M to about 2.0 M.

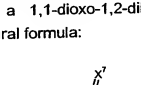
16. The activator solution of Claim 15, wherein the organic base is present in a concentration of about 0.01 M to about 2.0 M.

17. The activator solution of any one of Claims 10 to 16, wherein the organic base is pyridine, 3-methylpyridine, or N-methylimidazole.

18. The activator solution of any one of Claims 10 to 17, wherein the organic base is present in the same concentration as the 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one.

19. An activator solution according to any one of claims 10 to 18, wherein X^7 is O and p is 0.

20. A method of synthesizing an oligonucleotide using phosphoramidite chemistry comprising coupling a nucleoside or a nascent oligonucleotide having a free hydroxy or thiol group and a nucleoside phosphoramidite in the presence of a coupling agent, wherein the coupling agent is a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one represented by the following structural formula:



wherein:

p is 0 or an integer from 1 to 4;

X^7 is O or S;

R for each occurrence is a substituent.

21. The method of Claim 20, wherein each R independently is halo, a substituted or unsubstituted aliphatic group, $-NR^{11}R^{12}$, $-OR^{13}$, $-OC(O)R^{13}$, $-C(O)OR^{13}$, cyano, a substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclyl, -CHO, $-COR^{13}$, $-NHCOR^{13}$, a substituted or unsubstituted aralkyl, or $-SR^{13}$; or two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring; wherein:
R^{11} and R^{12} are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group; or together with the nitrogen to which they are attached form a heterocyclyl group; and
R^{13} is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group.

22. The method of Claims 20 or 21, wherein an organic base is present with the coupling agent during the coupling reaction.

23. The method of Claim 22, wherein the organic base is an amine.

24. The method of Claim 23, wherein the amine is a tertiary amine.

25. The method of Claim 24, wherein the tertiary amine is selected from the group

consisting of a trialkylamine, a substituted or unsubstituted N-alkylpyrrolidine, a substituted or unsubstituted N-arylpyrrolidine, a substituted or unsubstituted N-alkylpyrrole, a substituted or unsubstituted N-arylpyrrole, a substituted or unsubstituted N-alkylmorpholine, a substituted or unsubstituted N-arylmorpholine, a substituted or unsubstituted N-alkylpiperidine, a substituted or unsubstituted N-arylpiperidine, a substituted or unsubstituted N,N-dialkylpiperazine, a substituted or unsubstituted N,N-diarylpiperazine, a substituted or unsubstituted N-alkyl-N-arylpiperazine, substituted or unsubstituted quinuclidine, and a substituted or unsubstituted 1,8-diazabicyclo[5.4.0]undec-7ene.

26. The method of Claim 23, wherein the amine is a substituted or unsubstituted azaheterocyclyl compound.

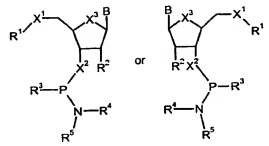
27. The method of Claim 26, wherein the azaheterocyclyl compound is selected from the group consisting of a substituted or unsubstituted pyrimidine, a substituted or unsubstituted 1-alkylpyrazole, a substituted or unsubstituted 1-arylpyrazole, a substituted or unsubstituted pyrazine, a substituted or unsubstituted N-alkylpurine, a substituted or unsubstituted N-arylpurine, a substituted or unsubstituted N-alkylpyrrole, a substituted or unsubstituted N-arylpyrrole, a substituted or unsubstituted pyridine, a substituted or unsubstituted N-alkylimidazole, a substituted or unsubstituted N-arylimidazole, a substituted or unsubstituted quinoline, a substituted or unsubstituted isoquinoline, a substituted or unsubstituted quinoxaline, a substituted or unsubstituted quinazoline, a substituted or unsubstituted N-alkylindole, a substituted or unsubstituted N-arylindole, a substituted or unsubstituted N-alkylbenzimidazole, a substituted or unsubstituted N-arylbenzimidazole, a substituted or unsubstituted triazine, a substituted or unsubstituted thiazole, a substituted or unsubstituted 1-alkyl-7-azaindole, a substituted or unsubstituted 1-aryl-7-azaindole, a substituted or unsubstituted pyrrolidine, a substituted or unsubstituted morpholine, a substituted or unsubstituted piperidine, and a substituted or unsubstituted piperazine.

28. The method of Claim 27, wherein the azaheterocyclyl compound is pyridine, 3-methylpyridine, or N-methylimidazole.

29. The method of any one of Claims 20 to 28, wherein the nucleoside phosphoramidite is a monomer.

30. The method of any one of Claims 20 to 28, wherein the nucleoside phosphoramidite is a dimer.

31. The method of any one of Claims 20 to 29, wherein the phosphoramidite is a 3'-phosphoramidite represented by one of the following structural formula:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or $-NR^{14}$-;

each X^3 is, independently, -O-, -S-, $-CH_2$-, or $-(CH_2)_2$-;

R^1 is an alcohol protecting group or a thio protecting group;

R^2 is -H, a substituted or unsubstituted aliphatic group, -F, $-OR^6$, $-NR^7R^8$, or $-SR^9$;

R^3 is $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^{10}$, $-SR^{10}$, $-O-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-O-CH_2CH_2-S(O)_2-CH_2CH_3$, $-O-CH_2CH_2-C_6H_4-NO_2$, $-S-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-S-CH_2CH_2-S(O)_2-CH_2CH_3$, or $-S-CH_2CH_2-C_6H_4-NO_2$.;

R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocyclyl group; and

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl, an hydroxy protecting group or $-(CH_2)_q-NR^{18}R^{19}$;

R^7 and R^8 are each, independently, H, a substituted or unsubstituted aliphatic group, or an amine protecting group; or

R^7 and R^8 taken together with the nitrogen to which they are attached are a heterocyclyl group; and

R^9 is H, a substituted or unsubstituted aliphatic group, or an thio protecting group;

R^{10} is for each occurrence is, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group or a substituted or unsubstituted aralkyl group,

R^{14} is -H, an alkyl group, an aryl group or an aralkyl group;

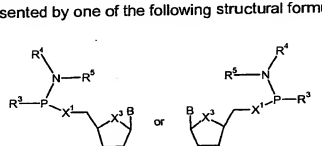
R^{18} and R^{19} are each, independently, -H, a substituted or unsubstituted aryl group, a substituted or unsubstituted heteroaryl group, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aralkyl group, a substituted or unsubstituted heteroaralkyl group or an amine protecting group; or

R^{18} and R^{19} taken together with the nitrogen to which they are attached form a heterocyclyl group; and

q is an integer from 1 to about 6; and

each B is, independently, -H, a natural or unnatural nucleobase, a protected natural or unnatural nucleobase, a heterocycle, or a protected heterocycle.

32. The method of any one of Claims 20 to 29, wherein the phosphoramidite is a 5'-phosphoramidite represented by one of the following structural formula:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or $-NR^{14}$-;

each X^3 is, independently, -O-, -S-, $-CH_2$-, or $-(CH_2)_2$-;

R^1 is an alcohol protecting group or a thio protecting group;

R^2 is -H, a substituted or unsubstituted aliphatic group, -F $-OR^6$, $-NR^7R^8$, or $-SR^9$;

R^3 is $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^{10}$, $-SR^{10}$, $-O-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-O-CH_2CH_2-S(O)_2-CH_2CH_3$, $-O-CH_2CH_2-C_6H_4-NO_2$, $-S-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-S-CH_2CH_2-S(O)_2-CH_2CH_3$, or $-S-CH_2CH_2-C_6H_4-NO_2$.;

R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocyclyl group; and

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or

unsubstituted aryl group, a substituted or unsubstituted aralkyl, an hydroxy protecting group or $-(CH_2)_q-NR^{18}R^{19}$;

R^7 and R^8 are each, independently, H, a substituted or unsubstituted aliphatic group, or an amine protecting group; or

R^7 and R^8 taken together with the nitrogen to which they are attached are a heterocyclyl group;

R^9 is H, a substituted or unsubstituted aliphatic group, or a thio protecting group;

R^{10} is for each occurrence is, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group or a substituted or unsubstituted aralkyl group,

R^{14} is -H, an alkyl group, an aryl group or an aralkyl group;

R^{18} and R^{19} are each, independently, -H, a substituted or unsubstituted aryl group, a substituted or unsubstituted heteroaryl group, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aralkyl group, a substituted or unsubstituted heteroaralkyl group or an amine protecting group; or

R^{18} and R^{19} taken together with the nitrogen to which they are attached form a heterocyclyl group; and

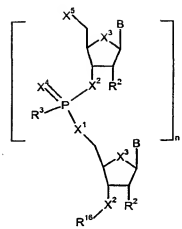
q is an integer from 1 to about 6; and

each B is, independently, -H, a natural or unnatural nucleobase, a protected natural or unnatural nucleobase, a heterocycle, or a protected heterocycle.

33. A method according to any one of Claims 20 to 32, wherein X^7 is O and p is 0.

34. A method according to any one of Claims 20 to 33, wherein the mole ratio of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one to nucleoside phosphoramidite is in the range of from about 0.2 : 1 to 5 : 1.

35. A method of condensing an N-mer oligonucleotide or a nucleoside represented by the following structural formula:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR^{14}-;

each X^3 is, independently, -O-, -S-, -CH_2-, or -$(CH_2)_2$-;

each X^4 is, independently, O or S;

X^5 is -OH or -SH;

each R^2 is, independently, -H, a substituted or unsubstituted aliphatic group, -F -OR^6, -NR^7R^8, or -SR^9;

each R^3 is, independently, -OCH_2CH_2CN, -SCH_2CH_2CN, a substituted or unsubstituted aliphatic group, -OR^{10}, -SR^{10}, -O-CH_2CH_2-$Si(CH_3)_2C_6H_5$, -O-CH_2CH_2-$S(O)_2$-CH_2CH_3, -O-CH_2CH_2-C_6H_4-NO_2, -S-CH_2CH_2-$Si(CH_3)_2C_6H_5$, -S-CH_2CH_2-$S(O)_2$-CH_2CH_3, or -S-CH_2CH_2-C_6H_4-NO_2.;

each R^6 is, independently, H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl, an hydroxy protecting group or -$(CH_2)_q$-$NR^{18}R^{19}$;

R^7 and R^8 are each, independently, H, a substituted or unsubstituted aliphatic group, or an amine protecting group; or

R^7 and R^8 taken together with the nitrogen to which they are attached are a heterocyclyl group; and

R^9 is H, a substituted or unsubstituted aliphatic group, or a thio protecting group;

R^{10} is for each occurrence is, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group or a substituted or unsubstituted aralkyl group,

each R^{14} is, independently, -H, an alkyl group, an aryl group or an aralkyl group;

R^{16} is a hydroxy protecting group, a thio protecting group, an amino protecting group, -$(CH_2)_q$-$NR^{18}R^{19}$, a solid support, or a cleavable linker attached to a solid support;

R^{18} and R^{19} are each, independently, -H, a substituted or unsubstituted aryl group, a

substituted or unsubstituted heteroaryl group, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aralkyl group, a substituted or unsubstituted heteroaralkyl group or an amine protecting group; or

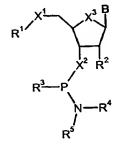
R^{18} and R^{19} taken together with the nitrogen to which they are attached form a heterocyclyl group; and

q is an integer from 1 to about 6;

each B is, independently, -H, a natural or unnatural nucleobase, a protected natural or unnatural nucleobase, a heterocycle, or a protected heterocycle; and

n is zero or a positive integer;

with a nucleoside phosphoramidite represented by the following formula:



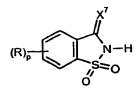
wherein R^2, R^3, X^1, X^2, X^3 and B are as previously defined:

R^1 is an alcohol protecting group or a thio protecting group; and

R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocyclyl group;

comprising the step of contacting the oligonucleotide or nucleoside with the nucleoside phosphoramidite and a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one represented by the following structural formula:



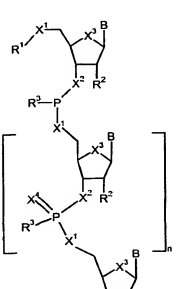
wherein:

p is 0 or an integer from 1 to 4;

X^7 is O and S;

R for each occurrence is a substituent;

thereby forming an (n+1) oligonucleotide having a 5'-terminal trivalent phosphorous linkage represented by the following structural formula:



36. The method of Claim 35, wherein each R independently is halo, a substituted or unsubstituted aliphatic group, $-NR^{11}R^{12}$, $-OR^{13}$, $-OC(O)R^{13}$, $-C(O)OR^{13}$, cyano, a substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclyl, -CHO, $-COR^{13}$, $-NHCOR^{13}$, a substituted or unsubstituted aralkyl, or $-SR^{13}$; or two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring; wherein:
R^{11} and R^{12} are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group; or together with the nitrogen to which they are attached form a heterocyclyl group; and
R^{13} is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group.

37. The method of Claim 35 or 36, wherein an organic base is present when the oligonucleotide or nucleoside is contacted with the phosphoramidite and the 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one.

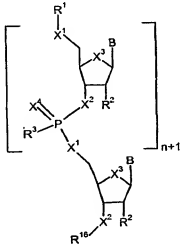
38. The method of Claim 37, wherein the organic base and the 1,1-dioxo-1,2-dihydro-$1\lambda^6$-benzo[d]isothiazol-3-one are present in the same concentration.

39. The method of Claim 37 or 38, wherein the concentration of the organic base and the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one is about 0.01 M to about 2 M.

40. The method of any one of Claims 35 to 39, wherein the mole ratio of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one to nucleoside phosphoramidite is in the range of from about 0.2 : 1 to 5 : 1.

41. The method of any one of Claims 35 to 40, further comprising the steps of:

a) contacting the (n+1) oligonucleotide having a 5'-terminal trivalent phosphorous linkage with an oxidizing or sulfurizing agent, thereby forming an oligonucleotide having a pentavalent phosphorous backbone represented by the following structural formula:

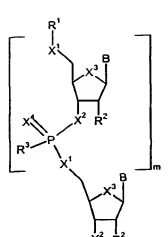


b) optionally capping X^5 groups which did not react with the phosphoramidite by reacting the X^5 groups with an acid chloride or an anhydride;

c) deprotecting the (n+1) oligonucleotide having a pentavalent phosphorous backbone by reacting it with a reagent to remove R^1;

d) optionally repeating the coupling step, oxidation or sulfurization step, the capping step and deprotection step one or more times.

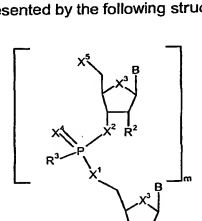
42. The method of Claim 41, wherein the oxidation or sulfurization step is the final step and the oligonucleotide is represented by the following structural formula:



wherein:

m is an integer greater than n.

43. The method of Claim 41, wherein the deprotection step is the final step and the oligonucleotide prepare is represented by the following structural formula:



wherein:

m is an integer greater than n.

44. The method of any one of Claims 35 to 43, wherein each R^2 is or $-OR^6$ and the oligonucleotide prepared is a ribonucleotide.

45. The method of any one of Claims 35 to 43, wherein each R^2 is -H and the oligonucleotide prepared is a deoxyribonucleotide.

46. The method of any one of Claims 35 to 43, wherein at least one of R^2 is H and at least one of R^2 is OR^6.

47. A method according to any one of Claims 35 to 46, wherein R^{16} is a cleavable linker attached to a solid support.

48. The method of Claim 41, wherein:

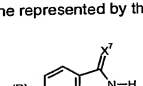
a) the oligonucleotide is oxidized after each coupling step and the oligonucleotide is a phosphodiester oligonucleotide;

b) the oligonucleotide is sulfurized after each coupling step and the oligonucleotide prepared is a phosphorothioate oligonucleotide; or

c) the oligonucleotide is oxidized after at least one coupling step and sulfurized after at least one coupling step and the oligonucleotide prepared is a chimeric oligonucleotide.

49. A method according to any one of Claims 35 to 48, wherein X^7 is O and p is 0.

50. A method of preparing a salt complex of an organic base and a 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one represented by the following structural formula:



wherein:

p is 0 or an integer from 1 to 4;

X^7 is O or S;

R for each occurrence is a substituent;

comprising the step of contacting the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one with an organic base, thereby causing a salt complex to form.

51. The method of Claim 50, wherein each R independently is halo, a substituted or unsubstituted aliphatic group, $-NR^{11}R^{12}$, $-OR^{13}$, $-OC(O)R^{13}$, $-C(O)OR^{13}$, cyano, a substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclyl, -CHO, $-COR^{13}$, $-NHCOR^{13}$, a substituted or unsubstituted aralkyl, or $-SR^{13}$; or two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring; wherein:

R^{11} and R^{12} are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group; or together with the nitrogen to which they are attached form a heterocyclyl group; and

R^{13} is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted

aryl group, or a substituted or unsubstituted aralkyl group.

52. The method of Claim 50 or 51, wherein the salt complex is crystalline.

53. The method of any one of Claims 50 to 52, wherein the contact occurs in the presence of an organic solvent.

54. The method of Claim 53, wherein the salt complex is separated from the organic solvent.

55. The method of Claim 53, further comprising the step of evaporating a portion of the organic solvent after adding the organic base.

56. The method of Claim 54, further comprising the step of washing the salt complex with ether or hexane after separating it from the organic solvent.

57. The method of Claim 53, wherein the organic solvent is acetonitrile.

58. The method of any one of Claims 50 to 57, wherein the organic base is pyridine, 3-methylpyridine, or N-methylimidazole.

59. The method of any one of Claims 50 to 58, wherein X^7 is O and p is 0.

60. A method for the synthesis of an oligonucleotide comprising coupling a nucleoside phosphoramidite, preferably a nucleoside 3'-phosphoramidite, with a nucleoside or nascent oligonucleotide comprising a free hydroxy group, preferably a free 5'-hydroxy group, in the presence of an activator, wherein the activator comprises a mixture of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and an N-alkylimidazole, preferably N-methylimidazole.

61. A method according to Claim 60, wherein the phosphoramidite comprises a moiety of formula $-P(OCH_2CH_2CN)N(CH(CH_3)_2)_2$.

62. A method according to either of Claims 60 or 61, wherein the concentration of each of the 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one and the N-alkylimidazole is from 0.1 to 0.25M.

63. A method according to any one of Claims 60 to 62, wherein the mole ratio of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one to N-alkylimidazole is from about 1 : 1 to

about 1 : 1.5 : 1.

64. A method according to any one of Claims 60 to 63, wherein the mole ratio of 1,1-dioxo-1,2-dihydro-1λ^6-benzo[d]isothiazol-3-one to phosphoramidite is from 0.5 : 1 to 2 : 1.

SEQUENCE LISTING

<110> Avecia Biotechnology, Inc. et al

<120> Immobilization of oligonucleotides onto solid supports

<130> SMC 60474-WO

<160> 1

<170> PatentIn version 3.1

<210> 1
<211> 18
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<213> Artificial Sequence

<220>
<223> Sequence prepared in Example 4

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18

INTERNATIONAL SEARCH REPORT

Inte al Application No
PCT/GB 02/03029

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H21/00 C07D275/06 C07D213/18 C07D233/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 034 534 A (MILSTEIN DAVID) 23 July 1991 (1991-07-23)	1-11, 15-19, 50-55, 58,59
Y	column 8, lines 14-15; column 9, line 24; column 11, lines 20-26; column 11, line 55 - column 12, line 37	50-57

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents:
'A' document defining the general state of the art which is not considered to be of particular relevance
'E' earlier document but published on or after the international filing date
'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
'O' document referring to an oral disclosure, use, exhibition or other means
'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 November 2002	29/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fitz, W

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INTERNATIONAL SEARCH REPORT

Int ial Application No
PCT/GB 02/03029

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with ind cation,where appropriate, of :he relevant passages	Relevant to claim No.
X	DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KAWADA, SEIGO ET AL: "Agrochemical germicides containing saccharin derivatives" retrieved from STN Database accession no. 79:74918 XP002220211	1-3, 6-11, 15-19, 50-54, 58,59
Y	abstract & JP 48 022628 B (KUMIAI CHEMICAL INDUSTRY CO., LTD.) 23 March 1973 (1973-03-23)	50-59
X	EP 0 061 434 A (MANETTI & ROBERTS ITALO BRIT) 29 September 1982 (1982-09-29)	1-3,6-8, 10,11, 15-18, 50-52,58
Y	page 3, lines 20-31; claim 2, page 2, line 24 - page 3, line 8	50-59
X	US 757 650 A (CHAS PFIZER &CO., INC.) 19 September 1956 (1956-09-19)	1-3, 6-11, 15-19, 50-54, 56,59
Y	page 2, column 1, line 102; page 2, column 2, lines 74-95	50-59
X	US 3 325 475 A (VACEK LUBOMIR C) 13 June 1967 (1967-06-13)	1-3,6,7, 9,50,51, 59
Y	the whole document	50-59
X	EP 0 274 023 A (BAYER AG) 13 July 1988 (1988-07-13)	1-7, 9-11, 13-16, 18,19, 50-55,59
Y	claim 1; page 14, lines 15-20; page 7, line 39	50-59
X	US 4 683 233 A (SALZBURG HERBERT ET AL) 28 July 1987 (1987-07-28)	1-7, 9-11, 13-16, 18,19, 50,51, 53-55,59
Y	tables; column 6, line 1; column 21	50-59

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INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB 02/03029

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	VILLALGORDO, J.M. ET AL.: "REACTIONS OF 2-MONOSUBSTITUTED 3-AMINO-2H-AZIRINES WITH NH-ACIDIC HETEROCYCLES" HELV. CHIM. ACTA, vol. 75, 1992, pages 2270-2282, XP002220208 page 2279, experimental part, paragraph 1.; page 2271, Scheme 3 ---	10-12, 15,16, 18,19
X	ABRAMOVITCH R.A. ET AL.: "NEW RING SYSTEMS FROM 1,2-BENZISOTHIAZOLE-1,1-DIOXIDES AND RELATED COMPOUNDS" TETRAHEDRON, vol. 52, no. 9, 1996, pages 3339-3354, XP002220209 page 3351, 3rd and 4th paragraph ---	10-12, 15,16, 18,19
Y	WO 99 62922 A (MANOHARAN MUTHIAH ;RAVIKUMAR VASULINGA T (US); SANGHVI YOGESH (US)) 9 December 1999 (1999-12-09) abstract ---	1-64
Y	MORIGUCHI: "SYNTHESIS AND PROPERTIES OF AMINOACYLAMIDO-AMP: CHEMICAL OPTIMIZATION FOR THE CONSTRUCTION OF AN N-ACYL PHOSPHORAMIDATE LINKAGE" J. ORG. CHEM., vol. 65, 2000, pages 8229-8238, XP002220210 the whole document, in particular: page 8231, Table 1, entry 6 -----	1-64

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty of claims 1-7,9-16,18,19,50-56,59. So many documents were retrieved that it is impossible to determine which parts of the claim(s) may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible.

Moreover, present claims 1-7,9-16,18-27,29-57,59 relate to an extremely large number of salt salt complexes, activator solutions and methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds, solutions and methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search report can be considered complete only for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the following organic bases: pyridine, 3-methylpyridine, N-methylimidazole, i.e. the salt complexes of claim 8, the activator solutions of claim 17, the methods of claims 28,58.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB 02/03029

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Int... ...nal Application No
PCT/GB 02/03029

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5034534	A	23-07-1991	NONE		
JP 48022628	B	07-07-1973	NONE		
EP 0061434	A	29-09-1982	AU	8133882 A	23-09-1982
			DD	202556 A5	21-09-1983
			DK	119782 A	19-09-1982
			EP	0061434 A1	29-09-1982
			ES	510525 D0	01-02-1983
			ES	8303384 A1	01-05-1983
			FI	820923 A	19-09-1982
			JP	57165380 A	12-10-1982
			NZ	199917 A	31-07-1985
			OA	7041 A	31-12-1983
			PT	74597 A ,B	01-04-1982
			US	4423053 A	27-12-1983
			ZA	8201744 A	26-01-1983
US 757650	A		NONE		
US 3325475	A	13-06-1967	NONE		
EP 0274023	A	13-07-1988	DE	3639901 A1	01-06-1988
			AU	8161387 A	26-05-1988
			BR	8706264 A	28-06-1988
			EP	0274023 A1	13-07-1988
			JP	63150269 A	22-06-1988
			US	4829063 A	09-05-1989
US 4683233	A	28-07-1987	DE	3430805 A1	19-09-1985
			AT	44207 T	15-07-1989
			AU	573787 B2	23-06-1988
			AU	3959585 A	12-09-1985
			BR	8501036 A	29-10-1985
			CA	1263841 A1	12-12-1989
			DE	3571188 D1	03-08-1989
			DK	105485 A	09-09-1985
			EP	0158074 A1	16-10-1985
			GR	850570 A1	03-07-1985
			HU	37334 A2	28-12-1985
			HU	196292 B	28-11-1988
			IL	74500 A	10-09-1989
			JP	60204707 A	16-10-1985
			PH	20699 A	24-03-1987
			ZA	8501723 A	27-11-1985
WO 9962922	A	09-12-1999	US	6274725 B1	14-08-2001
			AU	739468 B2	11-10-2001
			AU	4413599 A	20-12-1999
			CA	2330192 A1	09-12-1999
			EP	1084134 A1	21-03-2001
			JP	2002517404 T	18-06-2002
			WO	9962922 A1	09-12-1999

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